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Molecular Phylogenetic Investigation of U.S. Invasive *Tamarix*

JOHN F. GASKIN[1,2,3] and BARBARA A. SCHAAL[2]

[1]Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63110;
[2]Washington University in St. Louis, Campus Box 1137, One Brookings Drive, St. Louis, Missouri 63130;
[3]Current address: USDA Agricultural Research Service, Northern Plains Agricultural Research Laboratory; 1500 North Central Ave., Sidney, MT 59270. E-mail: jgaskin@sidney.ars.usda.gov

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ABSTRACT. *Tamarix* is a taxonomically difficult Old World genus that has become naturalized and invasive in the Americas and Australia. We examine the morphology and taxonomic history of 12 putative U.S. invasive *Tamarix* species, and investigate current invasions using chloroplast and nuclear sequence data. We test molecular phylogenetic hypotheses regarding the relationships of putative invasive taxa, and conclude that there are four invasive entities in the U.S., two of which are *T. aphylla* and *T. parviflora*. The sequence data also identify an invasive entity consisting of genetically indistinguishable *T. ramosissima* and *T. chinensis*, and another consisting of genetically indistinguishable *T. gallica* and *T. canariensis*. There is evidence of introgression between *T. ramosissima*, *T. canariensis*, and *T. gallica*, which is a likely source of confusion in the identification of some *Tamarix* invasions.

Invasions of non-native species into natural habitats are now considered, behind habitat destruction, the second largest ecological disaster worldwide (Wilson 1997). In the United States approximately 400 of the 972 plants and animals listed by the Endangered Species Act are at risk primarily due to competition with and predation by non-native species (Stein and Flack 1996). In addition to ecological damage, the economic cost of non-native species on the United States' agriculture, forestry, and public health is estimated to total $137 billion per year (Pimentel et al. 2000).

Within the U.S., the second worst plant invasion involves species of the Old World genus *Tamarix* L. (common name saltcedar or tamarisk; family Tamaricaceae) (Stein and Flack 1996). In the 1800's horticulturists intentionally imported up to twelve of the 54 species in the genus to the U.S. from southern Europe and/or Asia (Baum 1967; Crins 1989). Plants were used for shade and erosion control, and by 1987 a rapacious subset of the imports had overtaken more than 1,000,000 acres, primarily in the western U.S. (Brotherson and Field 1987). This infestation is expanding by 40,000 acres per year (Di Tomaso 1998), eroding the biodiversity in many natural areas, including major river systems and national parks. For information regarding the effects and control methods of *Tamarix* invasions see: Robinson 1965, Neill 1985, Kerpez and Smith 1987, Hughes 1993, Shafroth et al. 1995, Cleverly et al. 1997, Di Tomaso 1998, Duncan and McDaniel 1998, Gladwin and Roelle 1998, Glenn et al. 1998, Pitcairn 1998, Taylor and McDaniel 1998, and Zavaleta 2000.

Tamarix invasions have proved difficult to control. These plants cannot easily be killed by fire, herbicides, or cutting at ground level. Researchers at the United States Department of Agriculture (Agricultural Research Service) are currently searching for and testing candidate biological control insects as an alternative means of suppressing *Tamarix* invasions (DeLoach and Tracy 1997). A few of the *Tamarix* species invading the U.S. are morphologically distinct and easy to identify, however, there is ongoing controversy regarding the identity and corresponding native range of a majority of the invasive species (McClintock 1951; Crins 1989; Wilken 1993). Unfortunately, historical records rarely reveal precise geographic origins or specific identification of the introductions (Horton 1964). Improper characterization of the invasion could lead to searches for potential biological control agents outside the native range of the invasive, and thus result in ineffective or sub-optimal control agents. This situation stresses the importance of accurate taxonomic identification of invasive organisms, and underscores the potential power of molecular tools to complement and test traditional morphological hypotheses. Our objective is to investigate invasive *Tamarix* in the U.S. using molecular data, and compare our results with previous morphological analyses.

Tamarix was first monographed by Willdenow (1816), who described 16 species. Beginning with Decaisne (1835) taxonomic relationships were, and continue to be, based mostly on the morphology of the small nectary or androecial disc in the center of the flower. Bunge monographed the genus in 1852, identifying 51 species, and based much of his taxonomy on whether the racemes were produced on the previous year's woody branches (vernal) or on the current year's green branches (aestival). This character was considered diagnostically unreliable by Baum (1964), who later completed an exhaustive revision of the genus (Baum 1978) that has been complimented by Qaiser's (1983) work on the Pakistani species. Morphological treatments of the invasive U.S. *Tamarix* were prepared by McClintock (1951), Shinners (1957), Baum (1967), and Crins (1989),

TABLE 1. Putative U.S. *Tamarix* invasives compiled from McClintock (1951), Baum (1967), and Crins (1989), with taxonomic and morphological notes

T. africana: morphologically similar to *T. canariensis* and *T. gallica* in aestival floral form (Baum 1978)
T. aralensis: rarely cultivated, not extensively naturalized (Baum 1967)
T. aphylla: morphlogically dissimilar to all other U.S. *Tamarix*
T. canariensis: morphologically similar to *T. gallica* (Crins 1989)
T. chinensis: morphologically similar to *T. ramosissima* (Crins 1989)
T. gallica: morphologically similar to *T. canariensis* (Crins 1989)
T. juniperina: synonym of *T. chinensis* (Baum 1978)
T. parviflora: morphlogically dissimilar to all other invasive U.S. *Tamarix*
T. pentandra: synonym of *T. ramosissima* (Baum 1978)
T. ramosissima: morphologically similar to *T. chinensis* (Crins 1989)
T. tetrandra: U.S. invasive specimens with this name considered to be *T. parviflora* (Baum 1967)
T. tetragyna: naturalized in eastern U.S., not yet invasive (Crins 1989)

and a historical perspective of the U.S. invasion was written by Horton in 1964.

Tamarix is one of the more taxonomically difficult genera among the angiosperms (Baum 1978) and many taxa are indistinguishable in the vegetative state (Crins 1989). Hybridization may play a role in this taxonomic confusion (Rusanov 1949; Wilken 1993). The latest comprehensive revision of the genus by Baum contains three distinct sections, separated primarily by stamen number, petal length, androecial disk shape, and position of filament insertion on the androecial disk. These sections are further divided into nine series based on several morphological characters. Intermediate forms exist for many characters used in species identification, and these characters can often vary on a single individual from season to season (Rusanov 1949). All published chromosome counts for *Tamarix* are n = 12 (Baum 1978).

There have been at least 12 names applied to U.S. naturalized *Tamarix* (see Table 1). Note that *T. pentandra* Pall. is currently considered a synonym of *T. ramosissima* Ledeb. (Baum 1978); *T. juniperina* Bge. is considered a synonym of *T. chinensis* Lour. (Baum 1978); and invasive specimens determined as *T. tetrandra* Pall. ex M.B. emend. Willd. are now considered to be *T. parviflora* DC. (Baum 1967).

Baum's (1978) and Crins' (1989) studies agree that some characters are useful for segregating certain species, such as gross leaf morphology (vaginate vs. sessile), number of floral parts, and certain aspects of androecial disc morphology. The value of other characters, such as petal shape, presence or absence of hairs on the raceme rachis, and whether the filament is inserted under or from the side of the androecial disc are debated (Crins 1989). Both authors distinguish *T. aphylla* (L.) Karst. and *T. parviflora* from other U.S. invasives. The taxonomic ambiguity lies in distinguishing between species within the group *T. africana* Poir., *T. canariensis* Willd., and *T. gallica* L., and within the group *T. aralensis* Bge., *T. ramosissima*, and *T. chinensis*, which are differentiated by characters that may be variable within a species.

In this study we use DNA sequence data to identify species involved in the U.S. invasion, and to determine if the molecular data are congruent with the morphological distinctions that currently segregate taxa. We also test congruence of morphologically based sectional classifications and our molecular gene trees. Both nuclear and chloroplast markers are used to compare the evolutionary dynamics of two independent genomes, one maternally and one biparentally inherited, allowing investigation of putative hybridization within the genus.

MATERIALS AND METHODS

Sampling. *Tamarix ramosissima, T. parviflora, T. chinensis, T. canariensis, T. gallica,* and *T. aphylla* were collected from the western U.S., Argentina, and wild native populations across Eurasia and southern Africa. The remainder of the taxa analyzed include a broad selection of *Tamarix* species, and an individual from the sister genus *Myricaria*. The identities of all *Tamarix* were determined using Baum's (1978) morphological descriptions and keys. Collection and voucher information is listed in Appendix 1.

DNA Isolation, PCR Amplification and Sequencing. Fresh, silica dried tissue or samples from recent herbarium material were used for DNA extraction. Genomic DNA was isolated using a modified CTAB method (Hillis et al. 1996). PCR amplification of the chloroplast intergenic region between the *trn* S (GCU) and *trn* G (UCC) genes utilized the primer pair trn S (GCU) (GCCGCTTTAGTCCACTCAGC) and trn G (UCC) (GAACGAATCACACTTTTACCAC) of Hamilton (1999) with the following cycling conditions: 95°C (2 min); 30 cycles of 95°C (1 min), 55°C (1 min), 72°C (2 min); and then 32°C (5 min). PCR amplification of the internal transcribed spacer region (ITS) and intervening *5.8S* subunit of the *18S–26S* nuclear ribosomal DNA utilized either the primer pair ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) from Baldwin (1992), or our *Tamarix* specific primers ITSTX1F (ACTTGTTCACCGAAACACGG) and ITSTX4R (TAAGGCGCACGGCGTGATCC), with the following cycling conditions: 95°C (2 min); 30 cycles of 95°C (1 min), 58°C (1 min), 72°C (2 min); and then 32°C (5 min). A 50μL reaction was performed for each individual, and PCR products were purified by agarose gel electrophoresis followed by QIAquick Gel Extraction Kit (Qiagen, Valencia, California). Purified PCR template was sequenced using the dideoxy chain termination method with ABI PRISM® Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase (Perkin Elmer, Norwalk, Connecticut). Specimens were electrophoresed in an ABI 373A automated sequencer following manufacturer's instructions (Applied Biosystems, Foster City, California). Sequences generated in this study are available on GenBank.

Phylogenetic Analyses. Sequences were manually aligned using the software Se-Al (Rambaut 1996). The alignment is available upon request from the first author. Insertion/deletion events were treated as a fifth base, and multiple states (heterozygotes) were scored as polymorphisms. It is possible that the multiple copies of the nuclear marker in a single organism may not have identical evolutionary histories.

Parsimony analysis of the cpDNA data set was performed using PAUP* version 4.0b3 (Swofford 2000). Heuristic searches used TREE BISECTION RECONNECTION, COLLAPSE, and MULTREES options, with STEEPEST DESCENT not in effect. Analysis of the nuclear data set was similar to above except that the heuristic search used stepwise addition with the maximum number of trees set at 20,000 to limit computer search time. An additional 20 heuristic searches with random addition were performed with the maximum number of trees set at 5000 to explore alternative consensus topologies. Ten runs of 10,000 replicate "Fast Stepwise-addition" bootstrap analyses were conducted to assess clade support, with the lowest consensus bootstrap score of those runs recorded. The Templeton test (Templeton 1983) was used to compare alternative phylogenetic hypotheses. To perform this test, constraint topologies were created with monophyletic clades for the taxa of interest, leaving the rest of the tree unresolved. The data set was then reanalyzed under this constraint, and the resulting most parsimonious trees (maximum number =1000) were compared with up to five topographically distinct most parsimonious trees from the unconstrained analysis of the data set. The range of resultant P values from the Wilcoxon's signed rank test (Rolhf and Sokal 1995) was used to determine the statistical significance of the difference in length between the original and alternative topological hypotheses (significance at $P < 0.05$ in a one-tailed test).

Results

The cpDNA data set consists of 1001 aligned bases, of which 245 (24.5%) are variable and 66 (6.6%) are potentially phylogenetically informative, with 160 (0.2%) of the data matrix cells scored as missing. Excluding the outgroup, 89 (8.9%) sites are variable and 63 (6.3%) are potentially phylogenetically informative. The region between *trn* S (GCU) and *trn* G (UCC) was five times more variable than the region between *trn* L (UAA) and *trn* F (GAA) (see Taberlet et al. 1991), which contained only six phylogenetically informative sites out of 450bp (1.3%) in a previous analysis of *Tamarix* (J. Gaskin, unpubl. data). The cpDNA data set yields two most parsimonious trees 297 steps in length (RI = 0.95). With the uninformative characters excluded, CI = 0.82.

The nrDNA data set consists of 537 aligned bases, of which 235 (43.8%) are variable and 100 (18.6%) are potentially phylogenetically informative, with 679 (1.8%) of the data matrix cells scored as missing. Excluding the outgroup, 194 (36.1%) sites are variable and 78 (14.5%) of these are potentially phylogenetically informative. The nuclear data set yields a consensus tree 445 steps in length (RI = 0.83, maximum number of most parsimonious trees limited to 20,000). With the uninformative characters excluded, CI = 0.66. Twenty percent of the individuals are heterozygotic at one or more of their nrDNA bases. There are three consensus trees of the same length recovered when the search is repeated twenty times with random taxa addition. None of these alternate consensus trees has topologies inconsistent with the discussion below. The cpDNA and nrDNA gene trees are shown in Fig. 1. The clade names are arbitrary, with the chloroplast clades of interest designated by single letter names and the nuclear clades designated by two-letter names. Chloroplast clade "X" is not necessarily correlated with nuclear clade "XX".

Data sets are often combined to increase the resolution of the phylogeny, but an ILD test (incongruence length difference, or partition-homogeneity) (Farris et al. 1994) of the *Tamarix* data rejects the null hypothesis of congruence for the cpDNA and nrDNA data sets ($P=0.01$). In a Templeton test, constraining the cpDNA data set to the same topology that is generated by the combined data set results in a topology 33 steps longer than the best cpDNA tree. Therefore, the null hypothesis that these two topologies are statistically similar is rejected ($P < 0.0001$). Similarly, constraining the nrDNA data set to the same topology that is generated by the combined data set results in a topology 38 steps longer than the best nrDNA tree, and again the null hypothesis of these two topologies being statistically similar is rejected ($P < 0.0001$). Both the ILD and Templeton tests indicate that the two data sets should not be combined, and they will be treated below as separate sources of phylogenetic information.

Discussion

Congruence of Morphological and Molecular Data at Sectional Level. The three taxonomic sections based on morphology (Baum 1978) are not supported by the molecular analyses. Constraining the nrDNA data set to a topology in which all of the taxa are in monophyletic clades that reflect the sections sensu Baum (1978) results in topologies 57 steps longer than the original, and the null hypothesis of these topologies being statistically similar is rejected ($P < 0.0001$). Similarly, if the cpDNA data set is constrained to monophyletic sections sensu Baum, the resultant topologies are 28 steps longer than the original, and again the null hypothesis of the topologies being statistically similar is rejected ($P < 0.0001$). Thus, the morphological characters used to define the sections of *Tamarix* should be reevaluated.

Identification of U.S. Invasives. Invasive and horticultural *Tamarix* samples from the U.S. occur in five different clades of the cpDNA tree (see Fig. 1). The morphology of specimens found in these clades will be discussed below, along with their corresponding placement in the nrDNA tree and their relationships with U.S. invasive specimens found in other clades. The approximate distributions of these clades in the U.S. (J. Gaskin, unpubl. data) are shown in Fig. 2, but note that this is intended as a generalized schematic.

Clade A. The U.S. invasive *T. chinensis* and *T. ramosissima* have identical cpDNA sequences, except for specimen 84, which differs by one base mutation (*T. ramosissima* in clade E are horticultural and will be discussed later). These U.S. invasive *T. chinensis* and *T. ramosissima* are also found together in nrDNA clade

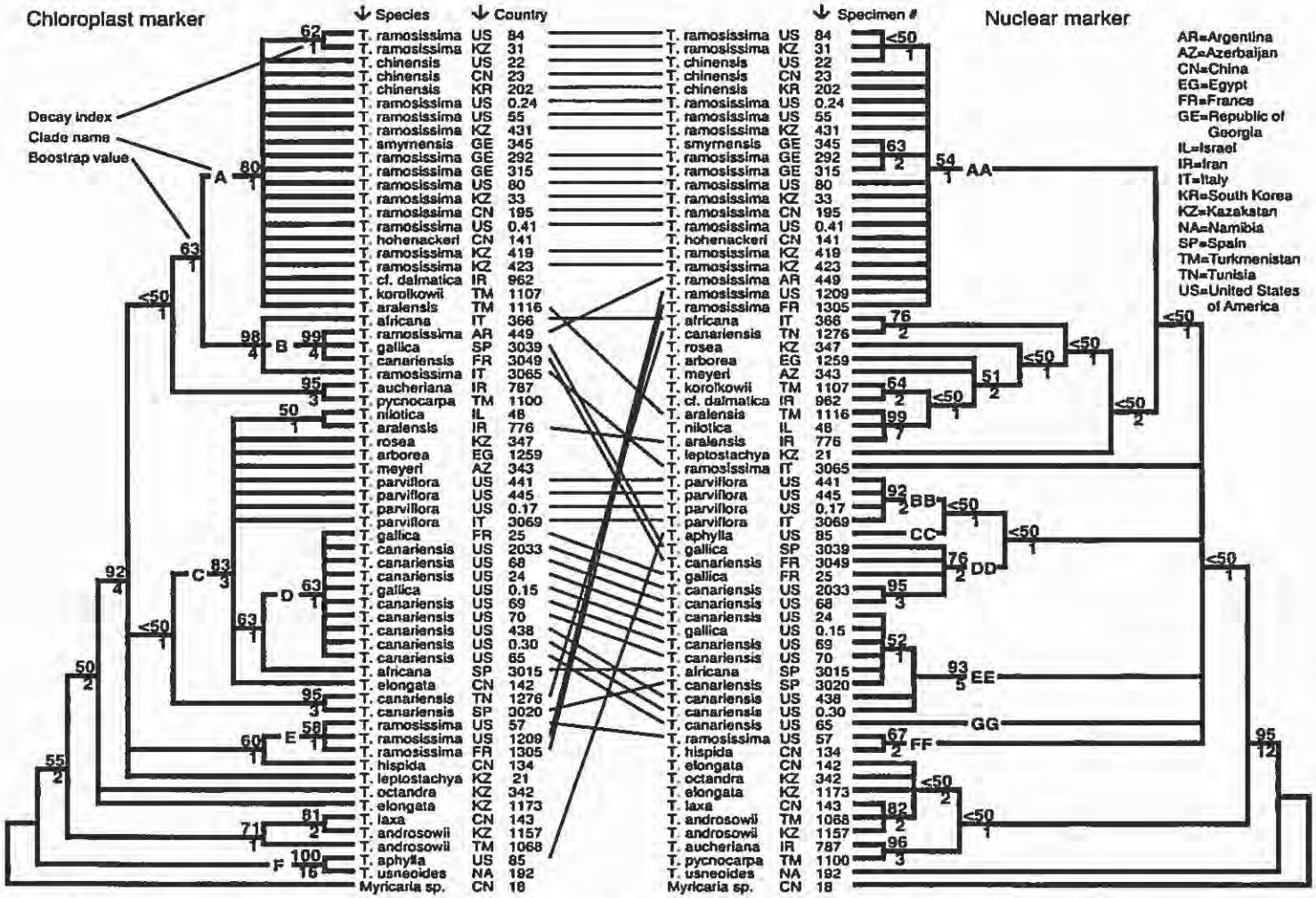

FIG. 1. Chloroplast and nuclear marker gene trees. On the left is one of two most parsimonious trees for the chloroplast marker (*trn* S-G), 297 steps in length, with an R.I. of 0.95 and a C.I. (excluding uninformative characters) of 0.82. On the right is a strict consensus of 20,000 most parsimonious trees for the nuclear marker (ITS 1–2), 445 steps in length, with an R.I. of 0.83 and a C.I. (excluding uninformative characters) of 0.66. Numbers above lines are bootstrap values and those below are decay indices. Taxa involved in the U.S. invasion have a line connecting them in both gene trees.

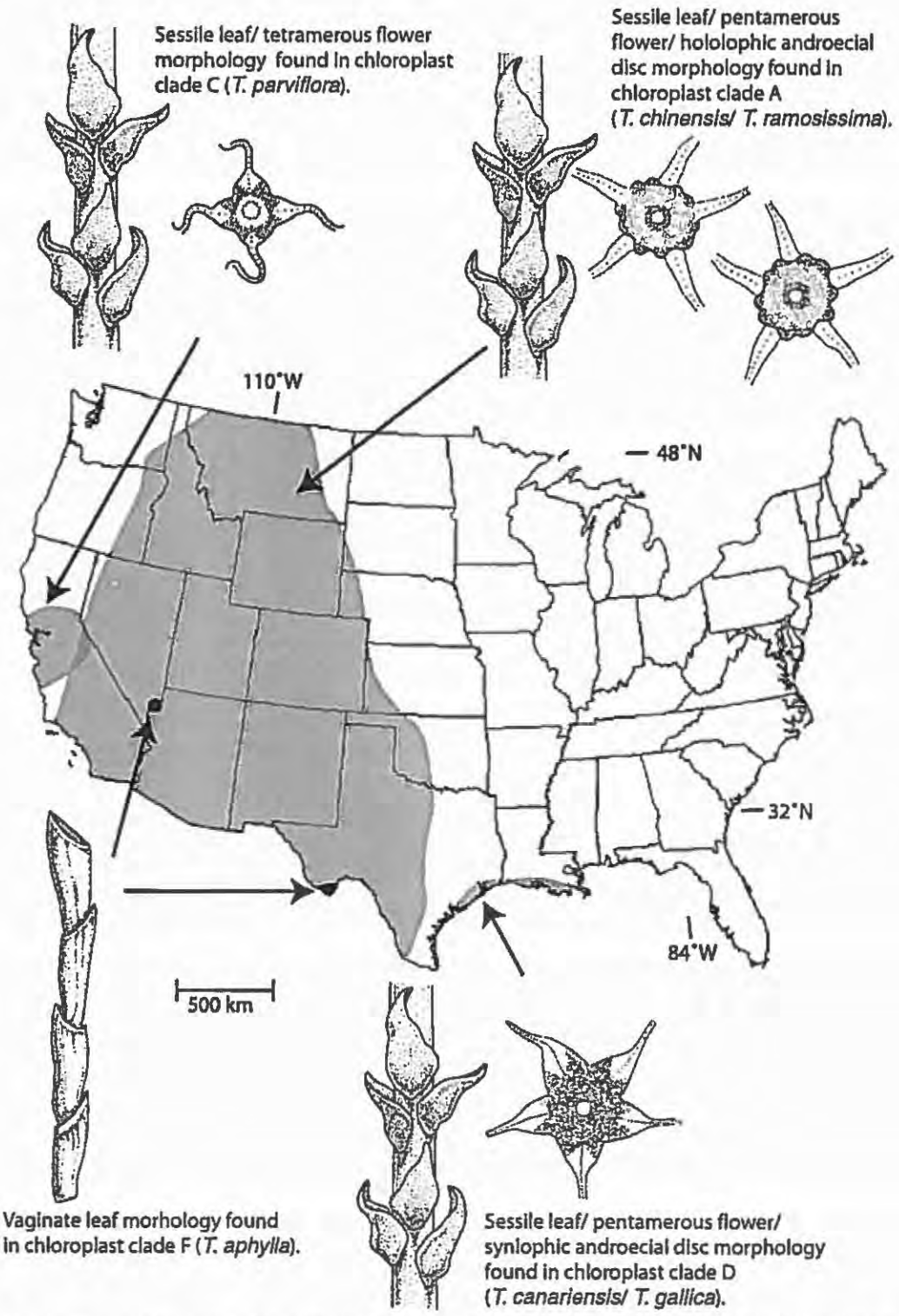

FIG. 2. Approximate distribution of four differing *Tamarix* morphologies in the United States of America. Illustrations include leaf morphology and androecial disc morphology.

AA. *Tamarix aralensis* (another putative U.S. invasive), *T. chinensis*, and *T. ramosissima* can be identified by their sessile leaves, pentamerous flowers and hololophic androecial discs (see Fig. 2). *Tamarix aralensis* is distinguished from *T. chinensis* and *T. ramosissima* by its caducous petals at the time of seed maturation. Additionally, *T. aralensis* is rarely cultivated and not extensively naturalized (Baum 1967). In Baum's revision (1978) *T. ramosissima* and *T. chinensis* are from different sections and series (section Tamarix series Gallicae vs. section Oligadenia series Laxae, respectively). They can be distinguished by eroded-denticulate vs. entire sepals, obovate vs. elliptic-ovate petals, halophilous vs. non-halophilous soil preference, 3–4 mm vs. 5–7 mm raceme width, and hypodiscal vs. hypo-peridiscal filament insertion (in aestival flowers), respectively. Crins (1989) claims that their morphology is similar, and that it is difficult to recognize these two taxa as different species, let alone members of different sections of the genus. *Tamarix chinensis* is native to China, Mongolia and Japan, while *T. ramosissima* is widespread from Turkey to Korea (Baum 1978). Invasive *T. ramosissima* and *T. chinensis* specimens are noted from many areas of the western U.S., and extend into Canada and Mexico. Given that both *T. chinensis* and *T. ramosissima* invasives are consistently in the same clade, these markers are not useful for distinguishing between these two species.

To determine if the U.S. *T. ramosissima* /*T. chinensis* from cpDNA clade A are a distinct invasive entity, they and the next closest invasive taxa (*T. parviflora* from clade C) in the cpDNA gene tree are constrained to a topology in which they are monophyletic. This results in a topology that is only four steps longer than the original topology, and thus the null hypothesis of these two topologies being statistically similar cannot be rejected (P = 0.1025). However, *T. ramosissima*/*T. chinensis* can distinguished from other invasives by the moderate bootstrap support (80%) of the cpDNA clade A and the consistent morphological combination of sessile leaves, pentamerous flowers and hololophic androecial discs (see Fig. 2).

CLADE C. The invasive and native *T. parviflora* specimens have identical cpDNA sequences and morphologies, and are found in the C clade. In the nrDNA gene tree, *T. parviflora* are all in clade BB. *Tamarix parviflora* is the only invasive that consistently has flowers with four sepals, petals and stamens. *Tamarix tetragyna* Ehrenb., another putative U.S. invasive, may have this form in native areas (Baum 1978), but Crins (1989) states that naturalized *T. tetragyna* can be distinguished from *T. parviflora* by its additional 1–4 antepetalous stamens. *Tamarix tetragyna* has only been naturalized on the Atlantic coast of Georgia (Crins 1989), and doesn't yet appear to be invasive in the U.S. *Tamarix parviflora*, native to southeastern Europe (Baum 1978) and planted extensively as an ornamental in the U.S., has invaded natural areas, especially in central California (J. Gaskin, pers. observ.).

Constraining the cpDNA to a topology in which *T. parviflora* and the next closest invasive taxa (*T. canariensis*, *T. gallica* from clade D) form a monophyletic clade results in a topology that is no longer than the original, thus, there is no statistical support for distinguishing between the two clades C and D. Similarly, constraining all of the *T. parviflora* of nrDNA clade BB to form a monophyly with the next closest invasive (*T. aphylla*), results in an alternative topology no longer than the original. However, there is support for considering *T. parviflora* as a distinct invasive entity due to its consistent and unique tetramerous floral morphology and the high bootstrap support (92%) for clade BB in the nrDNA gene tree.

CLADE D. The invasive *T. canariensis* and *T. gallica* all have identical cpDNA sequences and are found in clade D. In the nrDNA data set these invasives fall into separate clades, DD and EE, with a single specimen located on branch GG. Both *T. canariensis* and *T. gallica* are in clade DD, and *T. canariensis*, *T. gallica*, and *T. africana* are all in clade EE. Therefore, these three species cannot be distinguished using these molecular markers.

Tamarix africana, *T. gallica*, and *T. canariensis* all have sessile leaves, pentamerous flowers and synlophic androecial discs (see Fig. 2), and as a group may be easily distinguished from the other invasive species, though they are difficult to distinguish from one another, especially if the collected plant contains only aestival floral forms (Baum 1978, pg. 59). The morphological differences between vernal forms of these three species are: raceme width 5–9 mm (*T. africana*) vs. 4–5 mm (*T. canariensis* and *T. gallica*); papillose rachis of the raceme (*T. africana* and *T. canariensis*) vs. glabrous (*T. gallica*); and trullate-ovate to ovate petals (*T. africana*) vs. obovate (*T. canariensis*) vs. elliptic (*T. gallica*). Baum (1978) and others have placed these three similar species in two different sections of the genus. *Tamarix gallica* is placed in section Tamarix series Gallicae due to its narrower racemes (4–5 mm) and lack of papillae. *Tamarix canariensis* is in a different series (Leptostachyae) of the section Tamarix due to its papillose young growth, and *T. africana* is in a different section (Oligadenia) due to its broader racemes (5–9 mm). Crins (1989) notes that there is considerable morphological overlap between *T. canariensis* and *T. gallica*, and suggests that the relationships of these taxa be reevaluated.

All three species are sympatric in countries bordering the Mediterranean. Invasives in the U.S. are rarely identified as *T. africana*. None of our invasive samples match this species description, perhaps because the collections are all aestival forms of the plant. Crins (1989) and Baum (1967) note that *T. gallica* is rare in

the U.S. *Tamarix canariensis* is the most common determination of invasives with pentamerous, synlophic morphology, although all three species names are used for invasive specimens, especially along the gulf coast of Texas and Louisiana.

Constraining the nrDNA data set to a topology in which the invasive *T. canariensis* and *T. gallica* of nrDNA clades DD and EE are each in exclusive monophyletic clades results in an alternative topology 25 steps longer than the original, and the null hypothesis of these two topologies being statistically similar is rejected ($P < 0.0001$). Thus, clades DD and EE contain two distinct genotypes represented in the U.S. invasion, but it is not possible to assign a single species name or set of morphological characteristics to either one. This suggests that the morphological characters previously used to differentiate *T. canariensis* and *T. gallica*, such as papillae on the racemes and petal shape, are not reliable.

The inability of our molecular data to distinguish between *T. canariensis* and *T. gallica* may be due to these two species being the same taxon. In that case one expects all of the specimens, invasive and native, to reside in a single clade, which is not the case in either gene tree. Another possible explanation may be that the species are introgressing. For these species there is little correlation between the cpDNA and nrDNA (e.g. knowing a *T. canariensis* or *T. gallica* cpDNA sequence does not help in predicting the placement of that specimen on the nrDNA gene tree). Note how native specimens from nrDNA clade DD (25, 3039, and 3049) have cpDNA sequences from the two distinctly different clades, B and D. Constraining the cpDNA data set to a topology in which these three specimens form a monophyletic group results in an alternative topology 20 steps longer than the original. Therefore, the null hypothesis of these topologies being statistically similar is rejected ($P < 0.0001$). These distinct cpDNA evolutionary histories for specimens that share a single nrDNA history support a hypothesis of introgression (Whittemore and Schaal 1991; Soltis and Kuzoff 1995), even before being imported to the U.S. At this point introgression cannot be distinguished from ancestral lineage sorting without comparison to additional nuclear markers or a more resolved cpDNA gene tree. However, the historical and ongoing horticultural breeding programs for *Tamarix* in the U.S. and Eurasia support a hypothesis of hybridization.

CLADE E. The E clade of the cpDNA gene tree contains the three *T. ramosissima* horticultural specimens, two from the U.S. and one from France (not from the native range of *T. ramosissima*). The clade is weakly supported (bootstrap = 58%) and the specimens have identical sequences for this cpDNA marker. These horticultural *T. ramosissima* specimens and other *T. ramosissima* specimens from clades A and B are morphologically indistinct. But note that while these three horticultural *T. ramosissima* are identical for cpDNA sequence, two of them (1209 and 1305) have nrDNA sequences belonging to clade AA, and the third falls in the nrDNA clade FF, along with *T. hispida*. Constraining all of the horticultural *T. ramosissima* to form a monophyletic nrDNA clade results in an alternative topology that is only one step longer than the original, and we cannot reject the null hypothesis of these two topologies being statistically similar (P ranges from 0.7630 to 0.8273). Though there is no statistical support for distinct evolutionary histories of these various horticultural specimens, their placement into different (though weakly supported by bootstrap) clades warrants further investigation as potential hybrids.

CLADE F. The monophyletic cpDNA clade F containing *Tamarix aphylla* is well supported (bootstrap=100%, decay =16), and includes another vaginate leaved species, *T. usneoides*. *Tamarix aphylla* is easily distinguished from the other naturalized or invasive *Tamarix* by its vaginate leaves versus leaves with narrow bases (see Fig. 2). This species is native to xeric areas of the Middle East and Africa (Baum 1978). Widely used in horticulture both in the U.S. and its native range, it is now considered invasive in areas such as Lake Mead National Recreation Area, NV and Big Bend National Park, TX (DeLoach, pers. comm.). This species is also an aggressive invader in areas of Australia (Griffin et al. 1989).

We originally had analyzed seven *T. aphylla* specimens (six from the U.S. and one from Iran), all of which were identical for the cpDNA marker. Difficulty with ITS amplification resulted in only one nuclear sequence for this species. When *T. aphylla* is constrained in a monophyletic clade with the next closest invasive on the cpDNA gene tree (*T. ramosissima* in clade A), the alternative topology is 32 steps longer than the original, and therefore the null hypothesis of these two topologies being statistically similar is rejected ($P < 0.0001$). Thus, both morphological and molecular evidence support *T. aphylla* as a distinct invasive entity.

South American Invasion. An interesting example of possible introgression outside of the U.S. involves the invasive *T. ramosissima* from Argentina (449), which resides in the nrDNA clade AA along with all of the other invasive *T. ramosissima*. But, unlike the other invasive *T. ramosissima*, the Argentina specimen resides in clade B of the cpDNA along with native *T. gallica* and *T. canariensis*, not the expected clade A. Constraining the cpDNA data set to a topology in which the invasive specimen from Argentina and all of the other invasive *T. ramosissima* form a monophyletic clade results in an alternative topology 13 steps longer than the original. Therefore, the null hypothesis of these two topologies being statistically similar can be rejected ($P = 0.0003$). This provides strong support for specimen

449 being a hybrid between *T. ramosissima* and *T. canariensis* or *T. gallica*.

In conclusion, this analysis reveals that morphology within *Tamarix* does not always correlate with DNA sequence data. Baum's (1978) sectional classification of the genus is not statistically similar to either the cpDNA or nrDNA topologies, and future subgeneric classification of *Tamarix* must include molecular data.

Secondly, there is enough phylogenetic resolution to recognize four invasive *Tamarix* entities in the U.S. (*T. aphylla*, *T. parviflora*, *T. canariensis*/*T. gallica*, and *T. chinensis*/*T. ramosissima*). These molecular markers do not allow us to distinguish between *T. chinensis* and *T. ramosissima* morphologies sensu Baum (1978), and a study using markers that evolve more quickly is needed to further test the molecular distinctiveness of these two species. At this time, their placement into different sections is unsupported.

Additionally, the examples of statistically significant incongruence between the chloroplast and nuclear gene trees are possible evidence of hybridization within *Tamarix*, especially involving specimens of *T. canariensis*, *T. gallica*, and *T. ramosissima*. Hybridization may also be the cause of taxonomic difficulty in this genus. In the future we hope to examine the potential role of this hybridization in increased invasiveness and the creation of post-introduction novel genotypes.

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APPENDIX 1

Vouchers for exemplars used in DNA sequencing, and corresponding GenBank accesion numbers.

18. *Myricaria alopecuroides*. China, Wang Jian Feng 10 (USDA-GSWRL-BWCH). ITS AF484746, *Trn S-G* AF490774.
366. *Tamarix africana*. Italy, M. Olson s.n. (MO). ITS AF484760, *Trn S-G* AF490788.
3015. *Tamarix africana*. Spain, Gaskin 3015 (MO). ITS AF484805, *Trn S-G* AF490833.
1068. *Tamarix androsowii*. Turkmenistan, Gaskin 1068 (MO). ITS AF484757, *Trn S-G* AF490785.
1157. *Tamarix androsowii*. Kazakstan, Gaskin 1157 (MO). ITS AF484758, *Trn S-G* AF490786.
85. *Tamarix aphylla*. U.S., Gaskin 71 (MO). ITS AF484767, *Trn S-G* AF490795.
776. *Tamarix aralensis*. Iran, Gaskin 776 (MO). ITS AF484753, *Trn S-G* AF490781.
1116. *Tamarix aralensis*. Turkmenistan, Gaskin 1116 (MO). ITS AF484799, *Trn S-G* AF490827.
1259. *Tamarix arborea*. Egypt, Kirk 3 (MO). ITS AF484780, *Trn S-G* AF490808.
787. *Tamarix aucheriana*. Iran, Gaskin 787 (MO). ITS AF484762, *Trn S-G* AF490790.
0.3. *Tamarix canariensis*. U.S., DeLoach 00–30 (USDA-GSWRL-BWCH). ITS AF484782, *Trn S-G* AF490810.
24. *Tamarix canariensis*. U.S., DeLoach 3 (USDA-GSWRL-BWCH). ITS AF484752, *Trn S-G* AF490780.
65. *Tamarix canariensis*. U.S., Gaskin 34 (MO). ITS AF484801, *Trn S-G* AF490829.
68. *Tamarix canariensis*. U.S., Gaskin 36 (MO). ITS AF484802, *Trn S-G* AF490830.
69. *Tamarix canariensis*. U.S., Gaskin 35 (MO). ITS AF484803, *Trn S-G* AF490831.
70. *Tamarix canariensis*. U.S., Gaskin 37 (MO). ITS AF484804, *Trn S-G* AF490832.
438. *Tamarix canariensis*. U.S., DeLoach 00–01 (USDA-GSWRL-BWCH). ITS AF484778, *Trn S-G* AF490806.
1276. *Tamarix canariensis*. Tunisia, Kirk 2-Tunisia (MO). ITS AF484796, *Trn S-G* AF490824.
2033. *Tamarix canariensis*. U.S., Lievens 1293 (LSU) . ITS AF484800, *Trn S-G* AF490828.
3020. *Tamarix canariensis*. Spain, Gaskin 3020 (MO). ITS AF484806, *Trn S-G* AF490834.
3049. *Tamarix canariensis*. France, Gaskin 3049 (MO). ITS AF484808, *Trn S-G* AF490836.
22. *Tamarix chinensis*. U.S., J.L. Tracy 4 (USDA-GSWRL-BWCH). ITS AF484776, *Trn S-G* AF490804.
23. *Tamarix chinensis*. China, DeLoach 25 (USDA-GSWRL-BWCH). ITS AF484770, *Trn S-G* AF490798.
202. *Tamarix chinensis*. South Korea, Gaskin 202 (MO). ITS AF484771, *Trn S-G* AF490799.
962. *Tamarix* cf. *dalmatica*. Iran, Gaskin 962 (MO). ITS AF484794, *Trn S-G* AF490822.
142. *Tamarix elongata*. China, DeLoach s.n. (USDA-GSWRL-BWCH). ITS AF484777, *Trn S-G* AF490805.
1173. *Tamarix elongata*. Kazakstan, Gaskin 1173 (MO). ITS AF484764, *Trn S-G* AF490792.
0.15. *Tamarix gallica*. U.S., DeLoach 00–15 (USDA-GSWRL-BWCH). ITS AF484781, *Trn S-G* AF490809.
25. *Tamarix gallica*. France, R. Sobhian 13 (USDA-GSWRL-BWCH). ITS AF484775, *Trn S-G* AF490803.
3039. *Tamarix gallica*. Spain, Gaskin 3039 (MO). ITS AF484807, *Trn S-G* AF490835.
134. *Tamarix hispida*. China, DeLoach s.n. (USDA-GSWRL-BWCH). ITS AF484755, *Trn S-G* AF490783.
141. *Tamarix holuenackeri*. China, DeLoach s.n. (USDA-GSWRL-BWCH). ITS AF484779, *Trn S-G* AF490807.
1107. *Tamarix korolkowii*. Turkmenistan, Gaskin 1107 (MO). ITS AF484795, *Trn S-G* AF490823.
143. *Tamarix laxa*. China, DeLoach s.n. (USDA-GSWRL-BWCH). ITS AF484756, *Trn S-G* AF490784.
21. *Tamarix leptostachya*. Kazakstan, I.D. Mityaev 9

(USDA-GSWRL-BWCH). ITS AF484765, *Trn S-G* AF490793.

343. *Tamarix meyeri*. Azerbaijan, Gaskin 522 (MO). ITS AF484772, *Trn S-G* AF490800.

48. *Tamarix nilotica*. Israel, Cohen & Plitmann 48 (MO). ITS AF484749, *Trn S-G* AF490777.

342. *Tamarix octandra*. Kazakstan, V. Ivlev s.n. (MO). ITS AF484759, *Trn S-G* AF490787.

0.17. *Tamarix parviflora*. U.S., DeLoach 00–17 (USDA-GSWRL-BWCH). ITS AF484797, *Trn S-G* AF490825.

441. *Tamarix parviflora*. U.S., DeLoach 00–04 (USDA-GSWRL-BWCH). ITS AF484750, *Trn S-G* AF490778.

445. *Tamarix parviflora*. U.S., DeLoach 00–03 (USDA-GSWRL-BWCH). ITS AF484769, *Trn S-G* AF490797.

3069. *Tamarix parviflora*. Italy, Gaskin 3069 (MO). ITS AF484810, *Trn S-G* AY083839.

1100. *Tamarix pycnocarpa*. Turkmenistan, Gaskin 1100 (MO). ITS AF484763, *Trn S-G* AF490791.

0.24. *Tamarix ramosissima*. U.S., DeLoach 00–24 (USDA-GSWRL-BWCH). ITS AF484783, *Trn S-G* AF490811.

0.41. *Tamarix ramosissima*. U.S., DeLoach 00–41 (USDA-GSWRL-BWCH). ITS AF484784, *Trn S-G* AF490812.

31. *Tamarix ramosissima*. Kazakstan, I.D. Mityaev 19 (USDA-GSWRL-BWCH). ITS AF484768, *Trn S-G* AF490796.

33. *Tamarix ramosissima*. Kazakstan, I.D. Mityaev 20 (USDA-GSWRL-BWCH). ITS AF484788, *Trn S-G* AF490816.

55. *Tamarix ramosissima*. U.S., Gaskin 103 (MO). ITS AF484774, *Trn S-G* AF490802.

57. *Tamarix ramosissima*. U.S., Gaskin 100 (MO). ITS AF484754, *Trn S-G* AF490782.

80. *Tamarix ramosissima*. U.S., Gaskin 88 (MO). ITS AF484785, *Trn S-G* AF490813.

84. *Tamarix ramosissima*. U.S., Gaskin 69 (MO). ITS AF484747, *Trn S-G* AF490775.

195. *Tamarix ramosissima*. China, R. Crocker s.n. (USDA-GSWRL-BWCH). ITS AF484789, *Trn S-G* AF490817.

292. *Tamarix ramosissima*. Republic of Georgia, Gaskin 229 (MO). ITS AF484790, *Trn S-G* AF490818.

315. *Tamarix ramosissima*. Republic of Georgia, Gaskin 508 (MO). ITS AF484791, *Trn S-G* AF490819.

419. *Tamarix ramosissima*. Kazakstan, V. Ivlev s.n. (MO). ITS AF484792, *Trn S-G* AF490820.

423. *Tamarix ramosissima*. Kazakstan, V. Ivlev s.n. (MO). ITS AF484793, *Trn S-G* AF490821.

431. *Tamarix ramosissima*. Kazakstan, DeLoach s.n. (USDA-GSWRL-BWCH). ITS AF484748, *Trn S-G* AF490776.

449. *Tamarix ramosissima*. Argentina, Schulte 1 (MO). ITS AF484761, *Trn S-G* AF490789.

1209. *Tamarix ramosissima*. U.S., Gaskin 1209 (MO). ITS AF484786, *Trn S-G* AF490814.

1305. *Tamarix ramosissima*. France, Kirk 3-France (MO). ITS AF484787, *Trn S-G* AF490815.

3065. *Tamarix ramosissima*. Italy, Gaskin 3065 (MO). ITS AF484809, *Trn S-G* AF490837.

347. *Tamarix rosea*. Kazakstan, V. Ivlev s.n. (MO). ITS AF484751, *Trn S-G* AF490779.

345. *Tamarix smyrnensis*. Republic of Georgia, Gaskin 753 (MO). ITS AF484773, *Trn S-G* AF490801.

192. *Tamarix usneoides*. Namibia, M. Olson 717 (MO). ITS AF484766, Trn S-G AF490794.